3. THE CHEMISTRY OF CANNABIS

AMALA RAMAN[1] and ALPANA JOSHI[2]

[1] *Department of Pharmacy, King's College London, UK*
[2] *National Center for the Development of Natural Products, University of Mississippi, USA*

The phytochemistry of *Cannabis sativa* has been extensively researched and more than four hundred compounds belonging to a variety of phytochemical groups have been reported to occur in the plant. According to one estimate, over 7000 scientific papers had been published on cannabis, its constituents and their pharmacological activities by 1980 (Turner *et al.,* 1980). Many detailed descriptions of the chemistry of cannabis have been published over the years, such as those of Mechoulam (1973), Razdan (1973), Crombie and Crombie (1976), Schultes and Hoffman (1980), Harvey (1984) and a major review article dealing exhaustively with the phytochemistry of cannabis by Turner *et al.* (1980). In the present text, only the most important features of cannabis phytochemistry will be described; the interested reader is referred to one of the more extensive treatments listed above for greater detail. A further source of information is the annotated bibliography of cannabis covering the literature from 1964 published by Waller *et al.* in 1976 (Volume I) and 1982 (Volume II), updated with regular supplements from 1980 onwards (Waller *et al.,* 1980–1993/4).

The psychoactive effects of cannabis and its preparations have been ascribed in the main to the presence of tetrahydrocannabinols (THCs), in particular the compound Δ^9-tetrahydrocannabinol (Δ^9-THC), which was first isolated and identified in 1964 (Gaoni and Mechoulam, 1964a). Δ^9-THC is one of a group of mostly C21 compounds known as cannabinoids, which appear to be unique to *Cannabis sativa*. More recent studies have demonstrated that cannabinoids other than Δ^9-THC also exhibit a range of pharmacological activities (Formukong *et al.,* 1989). Cannabis also contains noncannabinoid compounds whose effects have not been so widely investigated. An important point regarding *Cannabis sativa* is that it shows considerable variation in its chemistry, as described later in this chapter.

CANNABINOID CONSTITUENTS OF CANNABIS

Numbering Systems for Cannabinoids

Over the years, at least 5 numbering systems have been used for cannabinoids (Eddy, 1965). Only two of these, however, are in widespread use (Figure 1). One is based on the formal chemical rules for numbering dibenzopyran type compounds, and is the system used by Chemical Abstracts. This system will be adopted in the present text. In the second system the cannabinoids are numbered as substituted monoterpenoids (based on p-cymene) due to their biogenetic origin. A reader scanning the literature on cannabis may therefore encounter a number of ways of referring to the same

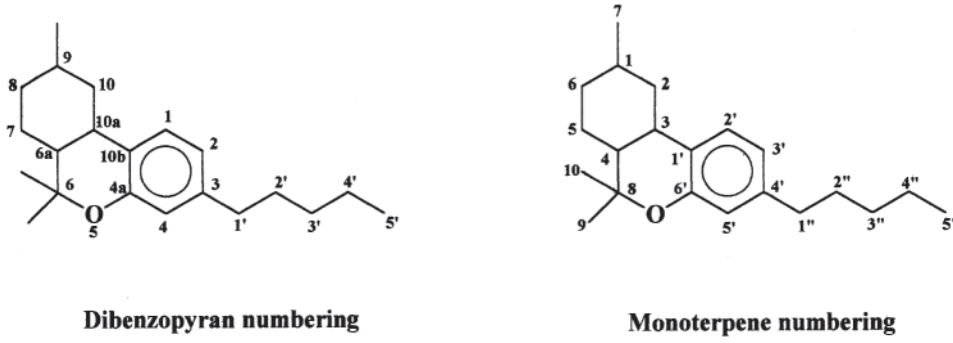

Figure 1 Two common numbering systems used for cannabinoids (Eddy, 1965)

compound. The major psychoactive component Δ^9-THC, for instance, may be described as either Δ^9-tetrahydrocannabinol (dibenzopyran system) or Δ^1-tetrahydrocannabinol (mono-terpenoid system). Similarly its minor structural isomer, Δ^8-tetrahydrocannabinol (dibenzopyran system), may be referred to as $\Delta^{1(6)}$-tetrahydrocannabinol (monoterpenoid system).

Structural Groups of Cannabinoids

The very large number of cannabinoids (over 60) known to occur in cannabis (Turner *et al.,* 1980) can be divided into a few main structural types as illustrated in Figure 2. These are the cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabicyclol (CBL), cannabielsoin (CBE), cannabinol (CBN), cannabinodiol (CBND) and cannabitriol (CBO) types. Variations on these basic types are fairly standard: presence or absence of a carboxyl group on the phenolic ring (at R^2 or R^4), a methyl, propyl or butyl side chain replacing the pentyl one (at R^3), or a methoxy group in place of one of the hydroxyl moieties. Some of the known compounds in each group are listed in Table 1 (from Turner *et al.,* 1980). For each type, the neutral compound with the pentyl side chain is normally referred to by the name and abbreviation listed above. In general, acid analogues have the letter A suffixed to the abbreviation, methyl ethers the letter M and methyl, propyl and butyl side chain analogues the suffix-C_n where n equals the number of carbons in the side chain. However, propyl analogues often have an abbreviation incorporating the letter V as their complete name usually includes the term "varin" e.g. cannabivarin, cannabi chromevarin (C_3 analogues of cannabinol and cannabichromene respectively).

Most natural cannabinoids have at least two chiral centres at carbons 10a and 6a (Figure 1). The absolute configuration at these centres was determined by Mechoulam and Gaoni (1967) for THC (10a R, 6a R) and CBD (10a S, 6a R). Further details regarding the isolation and absolute stereochemical configuration of the various cannabinoids in Figure 2 and Table 1 can be found in Turner *et al.* (1980).

In addition to the main cannabinoid groups described above, some usually very minor constituents belonging to related structural types have been shown to be present in cannabis. They include dehydrocannabifuran (DCBF), cannabifuran (CBF),

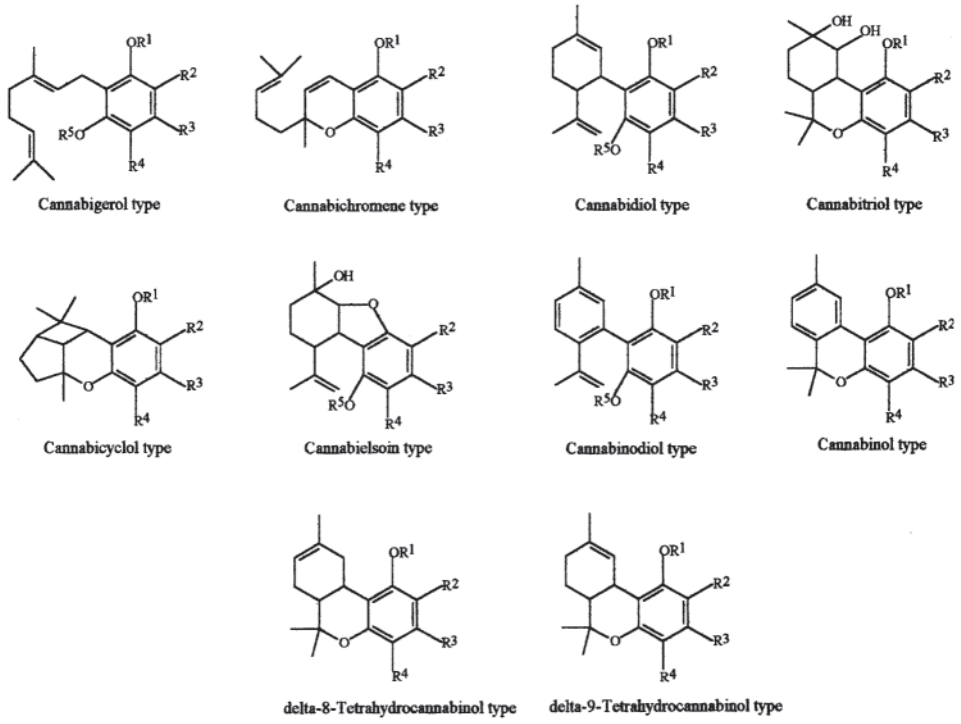

Figure 2 Main structural types of cannabinoids; see Table 1 for examples of compounds

cannabicitran (CBT), cannabichromanon (CBCN) and a dimeric cannabinoid formed by esterification of cannabidiolic acid with tetrahydrocannabitriol (Turner *et al.*, 1980). One of the most recent cannabinoids isolated from cannabis is cannabinerolic acid—the *trans* isomer of CBG (Taura *et al.*, 1995).

Chemical alteration of cannabinoids may occur during harvesting, storage or processing of cannabis preparations. CBN type compounds isolated from cannabis preparations are degradation products of the corresponding THC derivatives (Garret and Tsau, 1974; Turner and El Sohly, 1979; Harvey, 1985), and are not formed biosynthetically. The acid forms of THC are decarboxylated during storage probably by the agency of heat or light; this reaction occurs during smoking of cannabis preparations and in some analytical processes (Baker *et al.*, 1981). Δ^9-THC may isomerise to Δ^8-THC in the presence of strong acids (Mechoulam, 1973).

Biogenesis of Cannabinoids

Despite the interest in this group of compounds, surprisingly few actual experimental investigations have been conducted into the biogenesis of cannabinoids. Existing reports have variously involved either neutral compounds or the carboxylated forms. A general outline of the biogenetic origin of the cannabinoids, based on these studies as well as postulates, is depicted in Figure 3 (adapted from Harvey, 1984; Clarke, 1981; Schultes and Huffman, 1980; Turner and Mahlberg, 1985). Numbers in parentheses in this section refer to structures shown in Figure 3. For simplicity, only the acid forms are shown; the neutral cannabinoids commonly encountered in cannabis

Table 1 Examples of cannabinoids belonging to each of the main structural types shown in Figure 2

Cannabinoid type	*Name of cannabinoid*	R^1	R^2	R^3	R^4	R^5	*Abbreviation*
Cannabigerol							
	Cannabigerol	H	H	C_5H_{11}	H	H	CBG
	Cannabigerolic acid	H	COOH	C_5H_{11}	H	H	CBGA
	Cannabigerol monomethylether	H	H	C_5H_{11}	H	CH_3	CBGM
	Cannabigerolic acid monomethylether	H	COOH	C_5H_{11}	H	CH_3	CBGAM
	Cannabigerovarin	H	H	C_3H_7	H	H	CBG-C_3
	Cannabigerovarinic acid	H	COOH	C_3H_7	H	H	CBGA-C_3
Cannabichromene							
	Cannabichromene	H	H	C_5H_{11}	H	—	CBC
	Cannabichromenic acid	H	COOH	C_5H_{11}	H	—	CBCA
	Cannabichromevarin	H	H	C_3H_7	H	—	CBC-C_3
	Cannabichromevarinic acid	H	COOH	C_3H_7	H	—	CBCA-C_3
Cannabidiol							
	Cannabidiol	H	H	C_5H_{11}	H	H	CBD
	Cannabidiolic acid	H	COOH	C_5H_{11}	H	H	CBDA
	Cannabidiol monomethylether	H	H	C_5H_{11}	H	CH_3	CBDM
	Cannabidiorcol	H	H	CH_3	H	H	CBD-C_1
	Cannabidivarin	H	H	C_3H_7	H	H	CBDV, CBD-C_3
	Cannabidivarinic acid	H	COOH	C_3H_7	H	H	CBDVA, CBDA-C_3
	Cannabidiol-C_4	H	H	C_4H_9	H	H	CBD-C_4
Cannabitriol							
	Cannabitriol	H	H	C_5H_{11}	H	—	CBO
Cannabicyclol							
	Cannabicyclol	H	H	C_5H_{11}	H	—	CBL
	Cannabicyclolic acid	H	COOH	C_5H_{11}	H	—	CBLA
	Cannabicylovarin	H	H	C_3H_7	H	—	CBLV, CBL-C_3

Cannabielsoin							
	Cannabielsoin	—	H	C_5H_{11}	H	H	CBE
	Cannabielsoic acid A	—	COOH	C_5H_{11}	H	H	CBE acid A
	Cannabielsoic acid B	—	H	C_5H_{11}	COOH	H	CBE acid B
Cannabinodiol							
	Cannabinodiol	H	H	C_5H_{11}	H	H	CBND
	Cannabinodivarin	H	H	C_3H_7	H	H	CBVD, CBND-C_3
Cannabinol							
	Cannabinol	H	H	C_5H_{11}	H	—	CBN
	Cannabinolic acid	H	COOH	C_5H_{11}	H	—	CBNA
	Cannabinol monomethylether	CH_3	H	C_5H_{11}	H	—	CBNM
	Cannabiorcol	H	H	CH_3	H	—	CBN-C_1
	Cannabivarin	H	H	C_3H_7	H	—	CBV, CBN-C_3
	Cannabinol-C_4	H	H	C_4H_9	H	—	CBN-C_4
Δ^8-*Tetrahydrocannabinol*							
	Δ^8-Tetrahydrocannabinol	H	H	C_5H_{11}	H	—	Δ^8-THC
	Δ^8-Tetrahydrocannabinolic acid	H	COOH	C_5H_{11}	H	—	Δ^8-THCA
Δ^9-*Tetrahydrocannabinol*							
	Δ^9-Tetrahydrocannabinol	H	H	C_5H_{11}	H	—	Δ^9-THC
	Δ^9-Tetrahydrocannabinolic acid A	H	COOH	C_5H_{11}	H	—	Δ^9-THC acid A, Δ^9-THCA
	Δ^9-Tetrahydrocannabinolic acid B	H	H	C_5H_{11}	COOH	—	Δ^9-THC acid B, Δ^9-THCA
	Δ^9-Tetrahydrocannabiorcol	H	H	CH_3	H	—	Δ^9-THC-C_1
	Δ^9-Tetrahydrocannabiorcolic acid	H	H/COOH	CH_3	COOH/H	—	Δ^9-THCA-C_1
	Δ^9-Tetrahydrocannabivarin	H	H	C_3H_7	H	—	Δ^9-THCV, Δ^9-THC-C_3
	Δ^9-Tetrahydrocannabivarinic acid	H	H/COOH	C_3H_7	COOH/H	—	Δ^9-THCVA, Δ^9-THCA-C_3
	Δ^9-Tetrahydrocannabinol-C_4	H	H	C_4H_9	H	—	Δ^9-THC-C_4
	Δ^9-Tetrahydrocannabinolic acid-C_4	H	H/COOH	C_4H_9	COOH/H	—	Δ^9-THCA-C_4

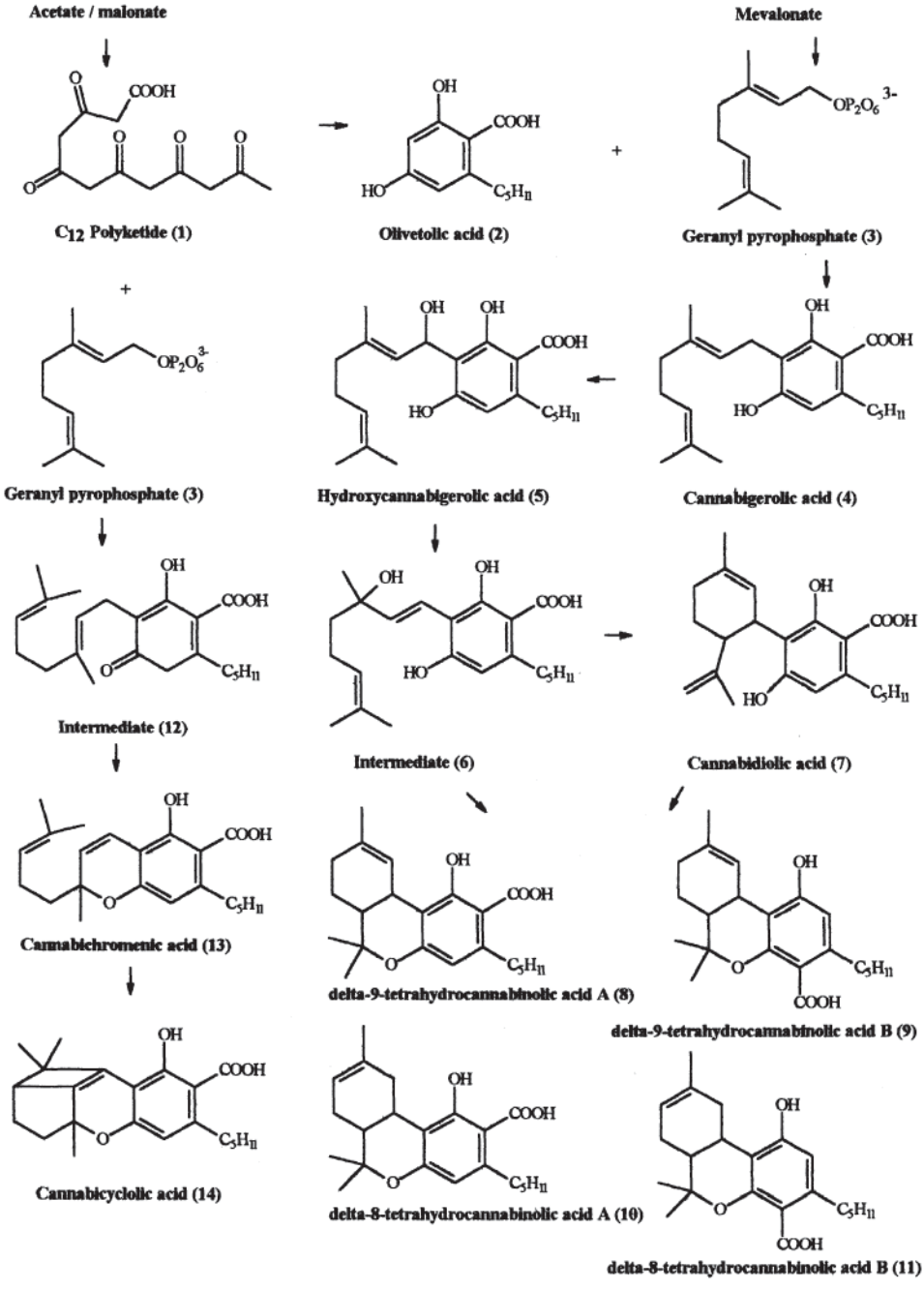

Figure 3 Proposed biogenetic pathway for the main cannabinoids

products may arise either by decarboxylation of the corresponding acids during harvesting and storage (Shoyama *et al.,* 1975) or by a biosynthetic pathway analogous to that shown, but involving the equivalent neutral precursors (Kajima and Piraux, 1982). In support of an independent pathway for neutral compounds, it has been observed that radiolabelled neutral precursors (olivetol and cannibigerol) are incorporated into THC and other neutral cannabinoids but not into THCA (Kajima and Piraux, 1982).

Some of the earliest articles on the biosynthesis of cannabinoids were published by Simonsen and Todd (1942), Farmilo *et al.* (1962) and Ni (1963) who proposed menthatriene, limonene and p-mentha-3, 8-diene-5-one repectively as terpene compounds which condensed with olivetolic acid, the precursor for the aromatic ring of the cannabinoids. However, it was Mechoulam and colleagues (Gaoni and Mechoulam, 1964b; Mechoulam and Gaoni, 1965; Mechoulam, 1970, 1973), who suggested the presently accepted route involving initial condensation of a phenolic compound, either olivetolic acid (**2**) or its decarboxylated analogue, olivetol with the terpene derivative geranyl pyrophosphate (**3**). This has since been supported by experimental studies (Shoyama *et al.,* 1975) in which malonate, mevalonate (precursors of olivetolic acid and geranyl pyrophosphate) and also geraniol and nerol were incorporated into THCA. CBC, however, appears to be formed by a different pathway; Turner and Mahlberg (1985) have shown that labelled olivetol administered to cannabis seedlings is incorporated only into CBG and THC, but not into CBC. This, and their finding that the developing plant first produces CBC and only later CBG and THC (Vogelmann *et al.,* 1988), implies the possible existence of two divergent pathways.

In the first route, CBCA (**13**) arises from combination of geranyl phosphate with a precursor of olivetolic acid (Turner and Mahlberg, 1985), possibly a C_{12} polyketide (**1**) derived from acetate/malonate (Shoyama *et al.,* 1975). However, there is also evidence that CBC can arise from CBG in some variants (Shoyama *et al.,* 1975). CBC and its acid form (**13**) are believed to be the precursors for CBL and CBLA (**14**) respectively.

In the second pathway, geranyl phosphate and olivetolic acid condense to form CBGA (**4**). Hydroxylation to hydroxycannabigerolic acid (**5**) is followed by rearrangement to an intermediate (**6**) which can then cyclise to form CBDA (**7**). Further cyclisation involving one or other of the phenolic hydroxyl groups leads to the potential (only three have actually been isolated from cannabis) formation of four isomeric THCAs (**8–11**) which vary in the position of the double bond and carboxylic acid group. However, Kajima and Piraux (1982) showed experimentally that CBD is not necessarily involved in THC biosynthesis. They suggest, in agreement with Turner and Hadley (1973), that a common intermediate (**6**) may give rise to either CBD or rearrange directly to THC. Variation in the levels of enzymes controlling these pathways may account for the chemical variation seen in different varieties of cannabis.

It is of interest to note that despite support for its involvement in cannabinoid biosynthesis, olivetol itself has not been reported to occur in cannabis. On the contrary, a prominent phenolic component of the glandular trichomes was found by Hammond and Mahlberg (1994) to be phloroglucinol (1,3,5-trihydroxybenzene), which they suggest may have some significance in cannabinoid biosynthesis.

Related components of cannabis, such as CBNA and its neutral analogue CBN, are not thought to be biogenetic products, but artefacts arising from the degradation of THCA and THC respectively (Harvey, 1984; Turner and El Sohly, 1979). Radiotracer studies show that the propyl side chain analogues of the cannabinoids do not arise by degradation of the pentyl side chain of the more common cannabinoids (Kajima and Piraux, 1982) and may involve a parallel biogenetic pathway.

Chemical Methods for Cannabinoid Synthesis

Interest in their pharmacological activity, as well as the need for reference materials for analytical purposes, has prompted the development of stereospecific synthetic methods for the production of cannabinoids in high yields. Synthetic processes for cannabinoids generally mirror the proposed biosynthetic sequence, involving the condensation of an optically active monoterpene with olivetol (5-pentylresorcinol). The monoterpene, reaction conditions and subsequent treatment of intermediates can be varied to obtain the desired cannabinoid product. Monoterpenes used by different researchers include p-mentha-2, 8-dien-1-ol (Petrzilka *et al.,* 1969), carene oxides (Razdan and Handrick, 1970), chrysanthenol (Razdan *et al.,* 1975), citral (El Sohly *et al.,* 1978) and p-menth-2-ene-1, 8-diol (Handrick *et al.,* 1979). Methods for the synthesis of Δ^9-THC and other cannabinoids have been reviewed in detail by Mechoulam *et al.* (1976), Crombie and Crombie (1976), and Razdan (1984).

NON-CANNABINOID CONSTITUENTS OF CANNABIS

Non-cannabinoid constituents isolated from various parts of the cannabis plant include a range of nitrogenous compounds (including alkaloids), sugars, sugar polymers, cyclitols, fatty acids, amino acids, proteins, glycoproteins, enzymes, hydrocarbons, simple alcohols, acids, aldehydes and ketones, steroids, terpenes, non-cannabinoid phenolic compounds, flavonoid glycosides, vitamins and pigments (Turner *et al.,* 1980). The majority of these compounds are found in many other species and are not unique to cannabis.

Some of the more unusual constituents of cannabis include an amide formed between p-hydroxy-*(trans)*-cinnamic acid and 2-(p-hydroxyphenyl)-ethylamine, which was isolated from the roots of Mexican cannabis (Slatkin *et al.,* 1971) and the spermidine alkaloids cannabisativine and anhydrocannabisativine isolated from the roots and aerial parts of various cannabis strains (Turner *et al.,* 1980). Non-cannabinoid phenolic compounds found in cannabis include spiro-indans (e.g. cannabispiran, cannabispirenone), dihydrostilbenes or bibenzyl compounds (e.g. canniprene) and cannabidihydrophenanthrene (Turner *et al.,* 1980). Additional non-cannabinoids isolated from cannabis since the publication of the review by Turner *et al.* (1980) include three new dihydrostilbenes (El-Feraly, 1984; El Sohly *et al.,* 1984)) and three new spiro-indans (El-Feraly *et al.,* 1986) either from hashish or leaves of cannabis, four phenyldihydronaphthalene lignanamides from cannabis fruits (Sakakibara *et al.,* 1991, 1992) and phloroglucinol glucoside from shoot latex (Hammond and Mahlberg, 1994). The volatile oil of indoor-grown cannabis has

been analysed and found to contain 68 components of which 57 were found to be known monoterpenes and sesquiterpenes (Ross and El Sohly, 1996).

Tris malonate acetylations and decarboxylations involving *p*-hydroxycinnamic acid have been reported to be involved in the biosynthesis of the dihydrostilbene (bibenzyl) compounds and flavones found in cannabis (Crombie *et al.,* 1988). The dihydrostilbenes are believed to be natural precursors of the spiro-indan compounds (El Sohly and Turner, 1982).

CHEMICAL VARIATION IN CANNABIS

Studies on a large number of cannabis plants originating from different parts of the world have led to the acceptance that a number of chemical races or "chemovars" of *Cannabis sativa* exist. These vary widely in their Δ^9-THC content and therefore psychoactive potency. The types cultivated for fibre production have very low levels of this compound, but show enhanced levels of its non-narcotic, biosynthetic precursor CBD. It has not been possible to correlate the chemovars directly with the different species or subspecies of *Cannabis* (e.g. *sativa, indica, ruderalis*) proposed by various authors (see Chapter 2), as these were primarily distinguished on morphological grounds. It is generally believed that the chemovars do not represent individual species, but owe their existence to centuries of cultivation and breeding for one of the two main products i.e. the intoxicant resin or the stem fibre.

A number of classification systems have been proposed to distinguish psychoactive and fibre strains of cannabis based on their cannabinoid composition (reviewed by Turner *et al.,* 1980). The first classification system, proposed by Grlic (1968), involved the use of a selection of chemical, spectroscopic, microbiological and pharmacological tests whose results were dependent on the levels of CBDA, CBD, Δ^9-THC and CBN in the sample. These markers were regarded as indicative of successive stages of "ripening" or subsequent decomposition of the resin. The more "ripe" samples (with higher levels of Δ^9-THC) were found to originate in tropical areas, commonly associated with production of intoxicant cannabis.

A few years later, a method based on quantitative analysis of specific cannabinoids was suggested by Waller and his colleagues (Waller and Scigliano, 1970; Fetterman *et al.,* 1971), in which the ratio of Δ^9-THC and its breakdown product CBN to the non-narcotic CBD was measured:

$$\text{Phenotype} = (\Delta^9\text{-THC} + \text{CBN}) / \text{CBD}.$$

Samples with ratios greater than 1 were classified as "drug type" and those with ratios below 1 as "fibre type" cannabis. Based on an examination of a large number of samples, Small and Beckstead (1973) further expanded the classification to four phenotypes:

Phenotype I: high (>0.3%) THC and low (<0.5%) CBD,
Phenotype II: at least 0.3% THC and high (>0.5%) CBD,
Phenotype III: relatively little THC and high (>0.5%) CBD,
Phenotype IV: plants consistently showing trace amounts of CBGM.

Turner *et al.* (1980) have outlined some of the limitations of the Waller and Small systems, which essentially only require the measurement of Δ^9-THC, CBD and CBN. These include the inadequate separation of CBD from CBC and CBV in the analytical systems employed at the time, the absence of CBD and CBC from cannabis of certain geographical origins, the presence of homologues (C_3 variants) in some samples which may contribute to psychoactive properties, and the influence of the age of the plant when analysed on its constituents, and consequently the phenotype to which it is assigned. They proposed that other cannabinoids (including C_3 homologues) should also be taken into consideration and derived the formula:

$$\text{Phenotype} = \frac{(\Delta^9\text{-THC} + \Delta^9\text{-THCV} + \text{CBN} + \Delta^8\text{-THC})}{(\text{CBDV} + \text{CBD} + \text{CBC} + \text{CBG} + \text{CBGM})}.$$

They suggest that the drug type (ratio > 1) and fibre type (ratio < 1) classification could be applied most reliably if the analyses were performed at regular intervals throughout the growing season of the plant, although this would not apply to confiscated samples.

Paris and Nahas (1984) have reviewed these classification systems and point out that the term "phenotype" is somewhat misleading as this generally refers to visible characteristics rather than genetic traits. They suggest classification into three chemical types, similar to phenotypes I–III of Small and Beckstead (1973) based on absolute content of THC and CBD rather than ratios:

(1) Drug type: THC>1 %, CBD=0,
(2) Intermediate drug type: THC>0.5%, CBD>0.5%,
(3) Fibre type: THC<0.25%, CBD>0.5%.

This classification into drug, fibre and intermediate types was first suggested by Turner (1980). In addition to the three main groups described above, Fournier *et al.* (1987), have reported a new chemotype of cannabis in which CBG (rather than CBD or Δ^9-THC) is the dominant cannabinoid. These chemotypes, however, cannot be considered as unique species or subspecies as it has been found that the variations in CBD and Δ^9-THC content among the plants is completely continuous, and further that individuals from strains belonging predominantly to one group may show characteristics of another (De Meijer *et al.*, 1992). A germplasm collection in which the predominant chemotype has been assessed has been established at Wageningen, the Netherlands (De Meijer and Van Soest, 1992).

Since the drug type and fibre type of cannabis have historically been associated with tropical and temperate regions of the world respectively, there has been considerable attention focussed on whether genetic or geographical factors govern the chemical nature of individual strains. Much of the work to date favours the primary importance of genetic factors in determining the cannabinoid profile of the plant. Fairbairn (1976), for example, reported that when seeds of specific cannabis strains representing either high Δ^9-THC or high CBD varieties were grown in a range of countries (UK, USA, Norway, Canada, Turkey, Thailand) all the plants from a particular batch showed a consistent CBD/Δ^9-THC pattern, although absolute content varied. Further evidence for genetic influence is that when high Δ^9-THC: low CBD

strains are crossed with low Δ^9-THC: high CBD varieties, the offspring show a cannabinoid content intermediate between the two (Clarke, 1981). That the local climate is not the primary influence on psychoactive potency is indicated by the successful outdoor cultivation of plants with relatively high Δ^9-THC content in Italy (Bertol and Mari, 1980; Avico *et al.,* 1985), Switzerland (Brenneisen and Kessler, 1987) and even the Danish island of Bornholm (Felby and Nielsen, 1985) which lies 55$N of the equator.

It has been suggested that over a number of generations, the chemical characteristics of a plant can alter to match more closely the type common to the area of cultivation. Bouquet (1951) reported that after several generations, plants grown in England and France from Indian seeds were indistinguishable from European (fibre) cultigens, whereas European varieties planted in Egypt as a source of fibre altered to low-fibre psychoactive forms. This may indicate the modifying influence of environmental factors, but the possibility of cross pollination with local strains during open cultivation cannot be ruled out. More recently a group in the United Kingdom has grown cannabis plants from seeds of diverse geographical origin under controlled conditions, and monitored their physical and chemical characteristics over four generations (Baker *et al.,* 1982, 1983; Taylor *et al.,* 1985). Marihuana samples prepared from the plants closely resembled the parent preparation even after four generations, and with a few exceptions within each group, the cannabinoid content was still typical of the profile obtained with the original source sample. A notable change in properties was that the THCA/THC ratios in the offspring were higher than in the source sample. This may be due to environmental factors; according to Mechoulam (1970), neutral cannabinoids are rarely found in cannabis grown in northern countries. However, it may also indicate the occurrence of decarboxylation during the preparation or storage of the original sample.

Genetic control of cannabinoid chemotypes is likely to be mediated via the synthesis of particular enzymes involved in cannabinoid biogenesis. In the proposed biosynthetic sequence (Figure 3), CBG is converted to an intermediate which can form either CBD or THC, and CBD may itself be converted to THC. Thus genetically controlled deficiencies in particular steps of the pathway can lead to CBG, CBD or THC dominant plants.

It is important to note that even though a plant may have the genetic capacity to express a particular enzyme, the environment could still influence the extent to which this occurs and therefore alter the cannabinoid content. In the study by Fairbairn (1976) described earlier, although the dominant cannabinoid remained unchanged in the different growth locations for a particular batch of seeds, variations were noted in the actual cannabinoid levels. In a group of Mexican drug type cannabis plants grown in Mississippi (Turner *et al.,* 1982), the CBC content was found to increase over a two year period. It was also noted that high temperatures and rainfall resulted in higher Δ^9-THC levels. Mahlberg and Hemphill (1983) have shown the importance of daylight in controlling Δ^9-THC and CBC levels. They found that red, blue and green filters had differing effects on the two cannabinoids, suggesting that the effect of light was being mediated via enzymes involved in their separate biosynthetic pathways. Pate (1983) has suggested that enhanced production of Δ^9-THC in regions of higher light intensity may indicate a protective role for the compound against the harmful effects of UVB radiation.

There is considerable evidence that as well as genetic and environmental factors, there is high inherent interplant variability between members of the same chemotype and even the same strain growing under identical conditions (Cortis *et al.,* 1985; De Meijer *et al.,* 1992). Daily and monthly fluctuations in the content of major cannabinoids have also been reported (Phillips *et al.,* 1970; Turner *et al.,* 1975).

Assessment of the chemical profile of a cannabis strain has been important for two main purposes—to distinguish drug and fibre chemotypes and to try to identify the geographical source of illicit samples of cannabis or cannabis products. Taking the first aspect, the recognition that fibre type cannabis generally has low levels of Δ^9-THC has been important in allowing countries to legislate for the cultivation of hemp and against the cultivation of narcotic cannabis. For instance, the maximum permitted Δ^9-THC content in fibre hemp is reported as 0.3% and 0.2% respectively for France (Bruneton, 1995) and the former USSR (De Meijer *et al.,* 1992). A review of the analytical methods that can be used to measure cannabinoid content is beyond the scope of this chapter, but a recent paper by Lehmann and Brenneisen (1995) who report comparative profiles of drug, fibre and intermediate types using high performance liquid chromatography (HPLC) coupled to photodiode array detection may be mentioned here.

A number of studies have examined the possibility of predicting the intoxicant potential of a particular cannabis plant or seed sample without the necessity of growing it to maturity. Independent studies carried out by Barni-Comparini *et al.* (1984) and Cortis *et al.* (1985) show that the cannabinoid profile of vegetative leaves even at an early stage in the plant's development is a good indication of its ultimate chemical characteristics. An attempt has been made to correlate the chemical characteristics of cannabis populations to some non-chemical traits (De Meijer *et al.,* 1992). Morphological features such as achene characteristics, stem width and internode length showed no correlation, but a weak association was found between psychoactive properties, leaflet width and date of anthesis. In another study, although variations were seen in the electrophoretic patterns of seed proteins from different cultivars, these could not be associated with the cannabinoid profile of the plant (De Meijer and Keizer, 1996). The potential use of random amplification of polymorphic DNA (RAPD) in the profiling of cannabis samples has been reported (Gillan *et al.,* 1995), but as yet no correlations to cannabinoid content have been made.

Cannabis strains that can be classified as drug type on the basis of their Δ^9-THC content, nevertheless show considerable variability in their overall phytochemical profile. Brenneisen and El Sohly (1988) have used high resolution gas-chromatography coupled to mass spectrometry (GC-MS), as well as HPLC, to examine the complex profiles of cannabis samples of various known geographical origins. Compounds appearing in the chromatographic profiles included both cannabinoids and non-cannabinoids, and samples from a common source showed similar characteristic peak patterns. Many of the diagnostically important peaks were found in the terpene region rather than amongst the cannabinoids. Certain components were only found in samples from particular sources e.g. allo-aromadendrene and tetrahydrocannbiorcol were characteristic of Mexican and Jamaican cannabis, whereas caryophyllene oxide (the terpene supposedly detected by sniffer dogs) was absent only in USA derived samples. However, only a limited number of samples were analysed from each source and further work is required to confirm these findings. Baker *et al.* (1980) examined

samples from various countries (between 5 and 150 samples from each source) by TLC and reported that although more than one type of product originated from a particular country, these could usually be visually and chemically distinguished. THV (Δ^9-THC-C_3) was common in illicit cannabis products from South Africa, Angola, Swaziland and Zimbabwe, sometimes exceeding Δ^9-THC in concentration, whereas samples from Ghana, Jamaica and Nigeria had low THV: Δ^9-THC ratios. CBG and CBC were common in Ghanaian samples. CBD was absent in samples from Kenya, Zambia, South Africa and Thailand (in the latter only THC and THCA were detected), whereas Moroccan, Pakistani and Lebanese hashish had significant levels of CBD. Indian cannabis was found to be highly variable in chemical composition, reflecting either the presence of many chemotypes under cultivation or the large size of the country. However, strict geographical patterns cannot be defined and are unlikely to be consistent over a long period of time due to exchange of seeds between countries, often as part of the illicit products transported.

REFERENCES

Avico, U., Pacifici, R. and Zuccaro, P. (1985) Variations of tetrahydrocannibinol content in cannabis plants to distinguish the fibre-type from drug-type plants. *Bull. Narc.,* **37**(4), 61–65.

Baker, P.B., Gough, L.A. and Taylor, B.J. (1980) Illicitly imported *Cannabis* products: some physical and chemical features indicative of their origin. *Bull. Narc.,* **32**(2), 31–40.

Baker, P.B., Gough, T.A. and Taylor, B.J. (1982) The physical and chemical features of *Cannabis* grown in the United Kingdom of Great Britain and Northern Ireland from seeds of known origin. *Bull. Narc. ,* **34**(1), 27–36.

Baker, P.B., Gough, T.A. and Taylor, B.J. (1983) The physical and chemical features of *Cannabis* grown in the United Kingdom of Great Britain and Northern Ireland from seeds of known origin—Part II: second generation studies. *Bull. Narc.,* **35**(1), 51–62.

Baker, P.B., Taylor, B.J. and Gough, T.A. (1981) The tetrahydrocannabinol and tetrahydrocannabinolic acid content of cannabis products. *J. Pharm. Pharmacol.,* **33,** 369–372.

Barni-Comparini, I., Ferri, S. and Centini, F. (1984) Cannabinoid level in the leaves as a tool for the early discrimination of cannabis chemovariants. *Forensic Sci. Int.,* **24,** 37–42.

Bertol, E. and Mari, F. (1980) Observations on cannabinoid content in *Cannabis sativa* L. grown in Tuscany, Italy. *Bull. Narc.,* **32**(4), 55–60.

Bouquet, R.J. (1951) Cannabis. *Bull. Narc.,* **3,** 14–30.

Brenneisen, R. and El Sohly, M.A. (1988) Chromatographic and spectroscopic profiles of *Cannabis* of different origins: Part I. *J. Forensic Sci.,* **33**(6), 1385–1404.

Brenneisen, R. and Kessler, T. (1987). Psychotrope Drogen 1. *Pharm. Acta Helv.,* **62**(5–6), 134–139.

Bruneton, J. (1995). Orcinols and Phloroglucinols. In *Pharmacognosy, Phytochemistry, Medicinal Plants,* Intercept Ltd, Hampshire, pp. 371–379.

Clarke, R.C.C. (1981) *Marijuana Botany,* And/or Press, Berkeley, California, p. 93 (cross-breeding); pp. 169–171 (cannabinoid biosynthesis).

Cortis, G., Luchi, P. and Palmas, M. (1985) Experimental cultivation of cannabis plants in the Mediterranean area. *Bull. Narc.,* **37**(4), 67–73.

Crombie, L. and Crombie, W.M.L. (1976) Chemistry of the cannabinoids. In J.D.P.Graham, (ed.), *Cannabis and Health,* Academic Press, London, pp. 43–76.

Crombie, L.W., Crombie, M.L. and Firth, D.F. (1988) Synthesis of bibenzyl cannabinoids,

hybrids of two biogenetic series found in *Cannabis sativa*. *J. Chem. Soc. Perkin Trans. I,* **5,** 1263–1270.

De Meijer, E.P.M. and Keizer, L.C.P. (1996) Patterns of diversity in *Cannabis*. *Genetic Resources and Crop Evolution,* **43,** 41–52.

De Meijer, E.P.M. and Van Soest L.J.M. (1992) The CPRO *Cannabis* germplasm collection. *Euphytica,* **62**(3), 201–211.

De Meijer, E.P.M., Van der Kamp, H.J. and Van Eeuwijk, F.A. (1992) Characterisation of *Cannabis* accessions with regard to cannabinoid content in relation to other plant characters. *Euphytica,* **62**(3), 187–200.

Eddy, N.B. (1965) *The Question of Cannabis,* Bibliography, United Nations Commission on Narcotic Drugs, E/CN7/49.

El-Feraly, F.S. (1984) Isolation, characterisation and synthesis of 3, 5, 4'-trihydroxybibenzyl from *Cannabis sativa*. *J. Nat. Prod.,* **47**(1), 89–92.

El-Feraly, F.S., El-Sherei, M.M. and Al-Muhtadi, F.J. (1986) Spiro-indans from *Cannabis sativa*. *Phytochem.,* **25**(8), 1992–1994.

El Sohly, H.N. and Turner, C.E. (1982) Constituents of *Cannabis sativa* L. XXII: isolation of spiro-indan and dihydrostilbene compounds from a Panamanian variant grown in Mississippi, United States of America. *Bull. Narc.,* **34**(2), 51–56.

El Sohly, H.N., Ma, G.E., Turner, C.E. and El Sohly, M.A. (1984). Constituents of *Cannabis sativa* XXV. Isolation of two new dihydrostilbenes from a Panamanian variant. *J. Nat. Prod.,* **47**(3), 445–452.

El Sohly, M.A., Boeren, E.G. and Turner, C.E. (1978) Constituents of *Cannabis sativa* L. An improved method for the synthesis of dl-cannabichromene. *J. Heterocyclic Chem.,* **15,** 699–700.

Fairbairn, J.W. (1976) The Pharmacognosy of Cannabis. In J.D.P.Graham, (ed.), *Cannabis and Health,* Academic Press, London, NY, San Fransisco, pp. 3–19.

Farmilo, C.G., Connell-Davis, T.W.M., Vandenheuval, F.A. and Lane, R. (1962) Studies on the chemical analysis of marihuana, biogenesis, paper chromatography, gas chromatography and country of origin . U.N. Secretariat Document, ST/SOA/Ser.s/7.

Felby, S. and Nielsen, E. (1985) Cannabinoid content of cannabis grown on the Danish island of Bornholm. *Bull. Narc.,* **37**(4), 87–94.

Fetterman, P.S., Keith, E.S., Waller, C.W., Guerrero, O., Doorenbos, N.J. and Quimby, M.W. (1971) Mississippi-grown *Cannabis sativa* L.: preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinol content versus age, sex and plant part. *J. Pharm. Sci.,* **60,** 1246–1249.

Formukong, E.A., Evans, A.T. and Evans, F.J. (1989) The medicinal uses of cannabis and its constituents. *Phytother. Res.,* **3**(6), 219–231.

Fournier, G., Richez-Dumanois, C, Duvezin, J., Mathieu, J.P. and Paris, M. (1987) Identification of a new chemotype in *Cannabis sativa:* cannabigerol-dominant plants, biogenetic and agronomic prospects. *Planta Med.,* **53**(3), 277–280.

Gaoni, Y. and Mechoulam, R. (1964a) Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.,* **86,** 1646–1647.

Gaoni, Y. and Mechoulam, R. (1964b). The structure and synthesis of cannabigerol a new hashish constituent. *Proc. Chem. Soc.,* March, 82.

Garrett, E.R. and Tsau, J. (1974) Stability of tetrahydrocannabinols I. *J. Pharm. Sci.,* **63,** 1563–1574.

Gillan, R., Cole, M.D., Linacre, A., Thorpe, J.W. and Watson, N.D. (1995) A comparison of *Cannabis sativa* by random amplification of polymorphic DNA (RAPD) and HPLC of cannabinoids: a preliminary study. *Science and Justice,* **35**(3), 169–177.

Grlic, L. (1968) A combined spectrophotometric differentiation of samples of Cannabis. *Bull. Narc.,* **20**(3), 25–29.

Hammond, C.T. and Mahlberg, P.G. (1994) Phloroglucinol glucoside as a natural constituent of *Cannabis sativa*. *Phytochem.,* **37**(3), 755–756.

Handrick, G.R., Uliss, D.B., Dalzell, H.C. and Razdan, R.K. (1979) Hashish: synthesis of (-)-delta-9-tetrahydrocannabinol (THC) and its biologically potent metabolite 3'-hydroxy-delta-9-THC. *Tetrahedron Lett.,* **8,** 681–684.

Harvey, D.J. (1984) Chemistry, metabolism and pharmacokinetics of cannabinoids. In G.H.Nahas, (ed.), *Marihuana in Science and Medicine,* Raven Press, NY, pp. 40–43.

Harvey, D.J. (1985) Examination of a 140 year old ethanolic extract of Cannabis: identification of new cannabitriol homologues and the ethylhomologue of cannabinol. In D.J.Harvey (ed.), *Marihuana '84: Proceedings of the Oxford Symposium on Cannabis,* IRL Press, Oxford, pp. 23–30.

Kajima, M. and Piraux, M. (1982) The biogenesis of cannabinoids in *Cannabis sativa*. *Phytochem.,* **21**(1), 67–69.

Lehmann, T. and Brenneisen, R. (1995) High performance liquid chromatographic profiling of cannabis products, *J. Liquid Chromatog.,* **18**(4), 689–700.

Mahlberg, P.G. and Hemphill, J.K. (1983) Effect of light quality on cannabinoid content of *Cannabis sativa* L. (Cannabaceae). *Bot. Gazette,* **144,** 43–48.

Mechoulam, R. (1970) Marijuana chemistry. *Science,* **168,** 1159–1166.

Mechoulam, R. (1973) *Marihuana: Chemistry, Pharmacology, Metabolism and Clinical Effects,* Academic Press, New York, London, pp. 1–99.

Mechoulam, R. and Gaoni, Y. (1965) A total synthesis of dl-?[1]-tetrahydrocannabinol, the active constituent of hashish. *J. Am. Chem. Soc.,* **87,** 3273–3275.

Mechoulam, R. and Gaoni, Y. (1967) Recent advances in the chemistry of hashish. In L.Zwxhmeister (ed.), *Fortschritte Chemisch Organischer Naturstoffe,* Vol. 25, Springer, Wien, pp. 175–213.

Mechoulam, R., McCallum, N.K. and Burstein, S. (1976) Recent advances in the chemistry and biochemistry of cannabis. *Chem. Rev.,* **76**(1), 75–112.

Ni, R. (1963) Part II. Studies on the biosynthesis of cannabinol and cannabidiol in *Cannabis sativa*. Thesis, University of Minnesota and University Microfilms Inc., Ann Arbor, Michigan.

Paris, M. and Nahas, G.G. (1984) Botany: The unstabilised species. In G.G.Nahas (ed.), *Marihuana in Science and Medicine,* Raven Press, NY, pp. 3–36.

Pate, D. (1983) Possible role of ultraviolet radiation in evolution of *Cannabis* chemotypes. *Econ. Bot.,* **37**(4), 396–405.

Petrzilka, T., Haefliger, W. and Sikemeier, C. (1969) Synthese von Haschisch-Inhaltsstoffen. *Helv. Chim. Acta,* **52,** 1102–1134.

Phillips, R., Turk, R., Manno, J., Jain, N. and Forney, R. (1970) Seasonal variation in Cannabinolic content of Indiana marihuana. *J. Forensic Sci.,* **15,** 191–200.

Razdan, R.K. (1973) Recent advances in the chemistry of cannabinoids. *Prog. Org. Chem.,* **8,** 78–101.

Razdan, R.K. (1984) Chemistry and structure activity relationships of cannabinoids: an overview. In S.Agurell, W.L.Dewey, and R.E.Willette, (eds.), *The Cannabinoids: Chemical, Pharmacological and Therapeutic Aspects,* Academic Press Inc, Orlando, Florida, pp. 63–78.

Razdan, R.K. and Handrick, G.R. (1970) Hashish: A stereospecific synthesis of (-)-delta-1-and (-)-delta-1(6)-tetrahydrocannabinols. *J. Am. Chem. Soc.,* **92,** 6061–6062.

Razdan, R.K., Woodland, L.R. and Handrick, G.R. (1975) A one-step synthesis of (-)-delta-1-tetrahydrocannabinol from crysanthenol. *Experientia,* **31**(1), 16–17.

Ross, S.A. and El Sohly, M.A. (1996) The volatile oil composition of fresh and air-dried buds of *Cannabis sativa*. *J. Nat. Prod.,* **59,** 49–51.

Sakakibara, I., Ikeya, Y., Hayashi, K. and Mitsuhashi, H. (1992) Three phenyldihydronaphthalene lignanamides from fruits of *Cannabis sativa*. *Phytochem.,* **31**(9), 3219–3223.

Sakakibara, L, Katsuhara, T., Ikeya, Y., Hayashi, K. and Mitsuhashi, H. (1991) Cannabisin A,

an arylnaphthalene lignanamide from fruits of *Cannabis sativa*. *Phytochem.,* **30**(9), 3013–3016.
Schultes, R.E. and Hoffman, A. (1980) *Botany and Chemistry of the Hallucinogens,* Charles C Thomas Publishers, Springfield, pp. 100–111.
Shoyama, Y., Yagi, M., Nishioka, I. and Yamauchi, T. (1975) Biosynthesis of cannabinoid acids. *Phytochem.,* **14,** 2189–2192.
Simonsen, J.L. and Todd, A.R. (1942) The essential oil from Egyptian hashish. *J. Chem. Soc.,* 188–191.
Slatkin, D.J., Doorenbos, N.J., Harris, L.S., Masoud, A.N., Quimby, M.W. and Schiff, P.L. (1971) Chemical constituents of *Cannabis sativa* L. root. *J. Pharm. Sci.,* **60,** 1891–1892.
Small, E. and Beckstead, H.D. (1973) Common cannabinoid phenotypes in 350 stocks of *Cannabis*. *Lloydia,* **36,** 144–165.
Taura, F., Morimoto, S. and Shoyama, Y. (1995) Cannabinerolic acid, a cannabinoid from *Cannabis sativa*. *Phytochem.,* **39**(2), 457–458.
Taylor, B.J., Neal, J.D. and Gough, T.A. (1985) The physical and chemical features of *Cannabis* grown in the United Kingdom of Great Britain and Northern Ireland from seeds of known origin—Part III: third and fourth generation studies. *Bull. Narc.,* **37**(4), 75–81.
Turner, C.E. and El Sohly, M.A. (1979) Constituents of *Cannabis sativa* L. XVI. A possible decomposition pathway of Δ^9-tetrahydrocannabinol to cannabinol. *J. Heterocyclic Chem.,* **16,** 1667–1668.
Turner, C.E. and Hadley, K. (1973) Constituents of *Cannabis sativa* L. II Absence of cannabidiol in an African variant. *J. Pharm. Sci,* **62**(2), 251–255.
Turner, C.E., El Sohly, H.N., Lewis, G.S., Lopez-Santibanez, I. and Carranza, I. (1982) Constituents of *Cannabis sativa* L., XX: the cannabinoid content of Mexican variants grown in Mexico and in Mississippi, United States of America. *Bull. Narc.,* **34**(1), 45–59.
Turner, C.E., El Sohly, M.A. and Boeren, E.G. (1980) Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *J. Nat. Prod.,* **43**(2), 169–234.
Turner, C.E., Fetterman, P.S., Hadley, K.W. and Urbanek, J.E. (1975) Constituents of *Cannabis sativa* L. X: Cannabinoid profile of a Mexican variant and its possible correlation to pharmacological activity. *Acta Pharm. Jugoslav.,* **25,** 7–16.
Turner, J.C. and Mahlberg, P.G. (1985) Cannabinoid synthesis in *Cannabis sativa L. Am. J. Bot.,* **72**(6), 911.
Turner, C.E. (1980) Marijuana research and problems: an overview. *Pharm. Int.,* **1,** 93–96.
Vogelmann, A.F., Turner, J.C. and Mahlberg, P.G. (1988) Cannabinoid composition in seedlings compared to adult plants of *Cannabis sativa*. *J. Nat. Prod.,* **51**(6), 1075–1079.
Waller, C.W. and Scigliano, J.A. (1970) The national marihuana program. Report to the commission of problems of drug dependence. *Natl. Acad. Sci NRC,* **4,** 28–32.
Waller, C.W., Johnson, J.J., Buelke, J. and Turner, C.E. (1976) *Marihuana: An Annotated Bibliography,* Vol. I, Macmillan, New York.
Waller, C.W., Baran, K.P., Urbanek, B.S. and Turner, C.E. (1980) *Marihuana: An Annotated Bibliography,* Supplement, Macmillan, New York.
Waller, C.W., Baran, K.P., Urbanek, B.S. and Turner, C.E. (1981) *Marihuana: An Annotated Bibliography,* Supplement, Macmillan, New York.
Waller, C.W., Nair, R.S., McAllister, A.F., Urbanek, B.S. and Turner, C.E. (1982) *Marihuana: An Annotated Bibliography,* Vol. II, Macmillan, New York.
Waller, C.W., Urbanek, B.S. and Wall, G.M. (1985–86; 1987–88; 1989–90; 1991–92; 1993–94) *Marihuana: An Annotated Bibliography,* Supplement, Macmillan, New York.
Waller, C.W., Urbanek, B.S., Wall, G.M., Mack, J.E. and Turner, C.E. (1982) *Marihuana: An Annotated Bibliography,* Supplement, Macmillan, New York.
Waller, C.W., Urbanek, B.S., Wall, G.M., Mack, J.E. and Turner, C.E. (1983–4) *Marihuana: An Annotated Bibliography,* Supplement, Macmillan, New York.